SYNTHESIS OF BRADYKININ ANALOGUES CONTAINING OPTICALLY-ACTIVE PIPECOLIC ACID

By

L. BALÁSPIRI, GY. PAPP, M. TÓTH, F. SIROKMÁN* and K. KOVÁCS

Institute of Medical Chemistry, University of Medicine, Szeged

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Seven structural analogues of bradykinin, containing optically-active L- and D-pipecolic acid**, were prepared and purified, and their physical constants were determined.

Slight changes in the structures of known peptide hormones by substituting amino acids of similar character for constituent amino acids are very important for investigation of the relationship between chemical structure and biological activity [1—3].

The amino acids substituted may be similar or not; they can be proteinogenic or nonproteinogenic. The known tissue hormone bradykinin

Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg

can be detected in the brain. Since this hormone contains three proline residues, it is easy to get more information by synthesis and biological study of bradykinin analogues containing one or more pipecolic acid residues in place of proline.

Up to now, numerous papers on the substitution of proline by optically-active pipecolic acid can be found in the literature of peptide chemistry. This synthesis may be achieved both classical and solid phase methods [4]. Among others, peptide analogues reported containing optically-active pipecolic acids include collagen models [5], oxytocin [6], angiotensin II [7], thyreotropin releasing hormone [8, 9], sequence-polypeptide [10] and model peptides [11]. Some others now under publication [12].

In order to fill gaps in the literature, special attention has been paid to the peptide chemical application of optically-active pipecolic acids [13]. Subsequently, the preparation of different protected and active derivatives of both L- and D-pipecolic acid was reported [14, 15] in detail. First, 3-L-pipecolic acid bradykinin appeared in the literature [16]; later, our research group reported 2-L- and 3-D-pipecolic acid

* Biological Center of the Hungarian Academy of Sciences, Szeged

** Nomenclature and abbreviations are those accepted by IUPAC—IUB for peptide chemistry: pip = pipecolic acid; Z = benzyloxycarbonyl; BOC = *t*-butyloxycarbonyl; OMe = methylester; ONb = = *p*-nitro-benzylester. Except for glycine, all proteinogenic amino acids are in the *L*-form.

Physical properties and analytical

Peptide	Method[2]	Purification[3]	Yield %
Z-Arg(NO_2)-L-Pip-Pro-Gly-Phe-Ser-L-Pip-Phe-Arg(NO_2)-OMe (**1**)	A	Aa	55
Arg-L-Pip-Pro-Gly-Phe-Ser-L-Pip-Phe-Arg (**2**)	A	Bd	44
Z-Arg(NO_2)-Pro-L-Pip-Gly-Phe-Ser-L-Pip-Phe-Arg(NO_2)-OMe (**3**)	A	Aa	52
Arg-Pro-L-Pip-Gly-Phe-Ser-L-Pip-Phe-Arg (**4**)	A	Bd	48
Z-Arg(NO_2)-D-Pip-Pro-Gly-Phe-Ser-Pro-Phe-Arg(NO_2)-ONb (**5**)	A	Ac	46
Arg-D-Pip-Pro-Gly-Phe-Ser-Pro-Phe-Arg(NO_2)-ONb (**6**)	A	Bd	70
Z-Arg(NO_2)-Pro-Pro-Gly-Phe-Ser-D-Pip-Phe-Arg(NO_2)-ONb (**7**)	A	Ac	50
Arg-Pro-Pro-Gly-Phe-Ser-D-Pip-Phe-Arg (**8**)	A	Bd	76
Z-Arg(NO_2)-Pro-Pro-Gly-Phe-Ser-L-Pip-Phe-Arg(NO_2)-ONb (**9**)	B	Ac	50
Arg-Pro-Pro-Gly-Phe-Ser-L-Pip-Phe-Arg (**10**)	B	Bd	84
Z-Arg(NO_2)-L-Pip-L-Pip-Gly-Phe-Ser-Pro-Phe-Arg-(NO_2)-OMe (**11**)	B	Aa	52
Arg-L-Pip-L-Pip-Gly-Phe-Ser-Pro-Phe-Arg (**12**)	B	Bd	39
Z-Arg(NO_2)-L-Pip-L-Pip-Gly-Phe-Ser-L-Pip-Phe-Arg(NO_2)-ONb (**13**)	B	Ac	52
Arg-L-Pip-L-Pip-Gly-Phe-Ser-L-Pip-Phe-Arg (**14**)	B	Bd	79

[1] all analogous are tabled
[2] A = stepwise condensation, B = fragment condensation
[3] A = silica gel column, B = Whatman CM 32 CMC-column, a = system 2, b = system 3, c = EtOAc-MeOH system, d = 0.01—0.5 M ammonium acetate
[4] A = acetic acid, D = dimethylformamide

bradykinin [17] and some fragments of further analogues [18, 19]. In this paper we describe the preparation of the following protected and free bradykinins: 2,7-L-pipecolic acid (**1—2**), 3,7-L-pipecolic acid (**3—4**), 2-D-pipecolic acid (**5—6**), 7-D-pipecolic acid (**7—8**), 7-L-pipecolic acid (**9—10**), 2,3-L-pipecolic acid (**11—12**) and 2,3,7-L-pipecolic acid bradykinins (**13—14**).

The first four analogues were synthesized in a stepwise manner, the others by a fragment condensation method. The biological data of these analogues will be published elsewhere [20].

Table I

data of the synthesized analogues[1]

M. p. °C	$[\alpha]_D$ c=1 T=24 °C[4]	Formula (Mol. weight)	Elemental analysis (N %) Calc. / Found / Amino acid analysis
128—137	−47° D	$C_{61}H_{83}O_{17}N_{17}$ (1326.5)	17.9 / 17.7
171—184	−80° A	$C_{58}H_{77}O_{11}N_{15}\times 3\ CH_3COOH$ (1268.3)	Arg 1.82 Pro 1.02 Pip 1.9 Gly 1.0 Pre 1.97 Ser 0.88
132—140	−50° D	$C_{61}H_{83}O_{17}N_{17}$ (1326.5)	17.9 / 17.7
176—186	−80° A	$C_{58}H_{77}O_{11}N_{15}\times 3\ CH_3COOH$ (1268.3)	Arg 1.8 Pro 1.02 Pip 1.8 Gly 1.0 Phe 1.97 Ser 0.83
138—140	−34° D	$C_{66}H_{84}O_{19}N_{18}$ (1433.5)	17.6 / 17.4
190—198	−58° A	$C_{57}H_{75}O_{11}N_{15}\times 3\ CH_3COOH$ (1254.3)	Arg 1.9 Pro 2.0 Pip 0.9 Gly 1.0 Phe 2.1 Ser 0.9
134—138	−34° D	$C_{66}H_{84}O_{19}N_{18}$ (1433.5)	17.6 / 17.4
190—200	−58° A	$C_{57}H_{75}O_{11}N_{15}\times 3\ CH_3COOH$ (1254.3)	Arg 1.84 Pro 1.86 Pip 0.8 Gly 1.0 Phe 1.88 Ser 0.91
140—150	−47° D	$C_{66}H_{84}O_{19}N_{18}$ (1433.5)	17.6 / 17.5
168—178	−80° A	$C_{57}H_{75}O_{11}N_{15}\times 3\ CH_3COOH$ (1254.3)	Arg 1.81 Pro 1.86 Pip 0.9 Gly 1.0 Phe 1.96 Ser 0.8
125—136	−48° D	$C_{61}H_{83}O_{17}N_7$ (1326.5)	17.9 / 17.6
165—175	−76° A	$C_{58}H_{77}O_{11}N_{15}\times 3\ CH_3COOH$ (1268.3)	Arg 1.82 Pro 0.9 Pip 1.8 Gly 1.0 Phe 1.98 Ser 0.88
117—124	−40° D	$C_{68}H_{88}O_{19}N_{18}$ (1461.6)	17.2 / 17.1
191—200	−82° A	$C_{59}H_{79}O_{11}N_{15}\times 3\ CH_3COOH$ (1282.4)	Arg 1.87 Pip 2.79 Gly 1.0 Phe 2.04 Ser 0.81

Experimental

Melting points were determined with a Kofler-block and optical rotations with a Zeiss-polarimeter. Amino acid analysis was performed on a Czechoslovakian HD-1200 E analyser after hydrolysis in 6 N HCl.

IR spectra were taken in KBr on a Unicam SP 200 instrument. TLC on Kiesel G plates was used for purity control and identification in the following systems:

1. *n*-butanol—acetic acid—water 4:1:1

2. ethyl acetate—pyridine—acetic acid—water 60:20:6:11
3. ethyl acetate—pyridine—acetic acid—water 80:20:6:11
4. ethyl acetate—pyridine—acetic acid—water 90:20:6:11
5. chloroform—methanol 8:2
6. chloroform—methanol—acetic acid 85:10:5.

The protected analogues were purified on a Merck Kieselgel 60 (0.063—0.2) column, eluted with system 2 or 3 or with ethyl acetate—methanol. The free peptides were purified by ion-exchange chromatography on a Whatman CM 32 carboxymethyl-cellulose column, eluted with a 0.01—0.5 M ammonium acetate gradient. Development of the TLC was carried out with ninhydrin, chlorine/*o*-toluidine and Sakaguchi reagent.

All data of described analogues can be found in Table I.

*Methyl benzyloxycarbonyl-arginyl(nitro)-*L*-pipecolyl-prolyl-glycyl-phenylalanyl-seryl-*L*-pipecolyl-phenylalanyl-nitroargininate* (**1**)

1.37 g (1.5 mmole) protected octapeptide ester [19] was treated with excess of trifluoroacetic acid at room temperature for 1 hour. The acid was evaporated in vacuum and the remaining trifluoroacetate of the octapeptide ester precipitated with ether, filtered, and dissolved in 8 ml dimethylformamide, cooled to −5 °C, and neutralized with triethylamine. 389 mg (1.65 mmole) benzyloxycarbonyl-(nitro)arginine was added, followed by 190 mg (1.65 mmole) N-hydroxysuccinimide (HOSu) and 310 mg (1.5 mmole) dicyclohexylcarbodiimide (DCCI). The reaction mixture was stirred at 0 °C for 4 hr, and then at room temperature for 24 hr. After filtering off the dicyclohexylurea, the filtrate was evaporated in vacuum, and the residue was triturated with ether, washed in turn with water, acetone and ether, and dissolved in methanol for column chromatography. The fractions were evaporated in vacuum, triturated in ether and dried.

*Arginyl-*L*-pipecolyl-prolyl-glycyl-phenylalanyl-seryl-*L*-pipecolyl-phenylalanyl-arginine triacetate* (**2**)

620 mg (**1**) was dissolved in dioxan—methanol 5:1, and 0.5 ml 2 N NaOH was added. After a 4-hr stirring at room temperature, 0.5 ml 2 N H_2SO_4 was added, and the mixture was evaporated in vacuum till half volume, poured into ice-water, filtered and washed with ether. The residual powder was dissolved in acetic acid—methanol 3:1, and the solution was hydrogenated in the presence of 10% Pd-C and Pd-black catalysts in a bubbling-apparatus at room temperature. The catalysts were filtered off and the filtrate was evaporated in vacuum. The residue was dissolved in start buffer (pH 5) and chromatographed. The main peak was collected and liophilized. The latter process was repeated twice from dilute acetic acid and water.

*Methyl benzyloxycarbonyl-arginyl(nitro)-prolyl-*L*-pipecolyl-glycyl-phenylalanyl-seryl-*L*-pipecolyl-phenylalanyl nitroargininate* (**3**)

1.0 g (1.0 mmole) protected octapeptide ester [19] was treated with trifluoroacetic acid, and the trifluoroacetate of the octapeptide ester, coupled with benzyloxycarbonyl(nitro)arginine in the presence of HOSu and DCCI, was worked up and chromatographed as described for (**1**).

*Arginyl-prolyl-*L*-pipecolyl-glycyl-phenylalanyl-seryl-*L*-pipecolyl-phenylalanyl-arginine triacetate* (**4**)

150 mg (**3**) was hydrolyzed, hydrogenated, chromatographed and worked up as described for (**2**).

*p-Nitrobenzyl benzyloxycarbonyl-arginyl(nitro)-*D*-pipecolylprolyl-glycyl-phenylalanyl-seryl-prolyl-phenylalanyl-nitro-nitroargininate* (**5**)

1.2 g (1.0 mmole) protected octapeptide ester [19] was treated with trifluoroacetic acid, and the trifluoroacetate of the octapeptide ester, coupled with benzyloxycarbonyl-(nitro)arginine in the presence of HOSu and DCCI, was worked up as described for (**1**), chromatographed in ethyl acetate—methanol and worked up similarly.

*Arginyl-*D*-pipecolyl-prolyl-glycyl-phenylalanyl-seryl-prolyl-phenylalanyl-arginine triacetate* (**6**)

143 mg (**5**) was hydrogenated as described for (**2**) (but only in the presence of Pd-black catalyst) and the crude product was chromatographed and worked up similarly.

*p-Nitrobenzyl benzyloxycarbonyl-arginyl(nitro)-prolyl-prolyl-glycyl-phenylalanyl-seryl-*D*-pipecolyl-phenylalanyl-nitroargininate* (**7**)

1.2 g (1.0 mmole) protected octapeptide ester [19] was treated with trifluoroacetic acid, and the trifluoroacetate of the octapeptide ester, coupled with benzyloxycarbonyl-(nitro)arginine in the presence of HOSu and DCCI, was worked up as described for (**1**), with chromatography in ethyl acetate—methanol as for (**5**).

*Arginyl-prolyl-prolyl-glycyl-phenylalanyl-seryl-*D*-pipecolyl-phenylalanyl-arginine triacetate* (**8**)

380 mg (**7**) was hydrogenated, chromatographed, and worked up as described for (**6**).

p-Nitrobenzyl benzyloxycarbonyl-arginyl(nitro)-prolyl-glycyl-phenylalanyl-seryl-L-pipecolyl-phenylalanyl-nitroargininate (**9**)

1.54 g (1.5 mmole) protected hexapeptide ester [19] was dissolved in acetic acid — HBr. After 1 hr the solution was evaporated in vacuum, solidified with ether, washed with ether and dried. The crude product hydrobromide of the hexapeptide ester was dissolved in dimethylformamide, cooled to −15 °C and neutralized with N-methyl morpholine. From 840 mg benzyloxycarbonyl-arginyl(nitro)-prolyl-proline the azide was prepared in situ at −15 °C. The two cooled solutions were poured together and stirred for 48 hr at 0 °C, and then filtered, evaporated in vacuum, dissolved for chromatography, and worked up as described for (**5**).

Arginyl-prolyl-prolyl-glycyl-phenylalanyl-seryl-L-pipecolyl-phenylalanyl-arginine triacetate (**10**)

800 mg (**9**) was hydrogenated, chromatographed, and worked up as described for (**6**).

Methyl benzyloxycarbonyl-arginyl(nitro)-L-pipecolyl-L-pipecolyl-glycyl-phenylalanyl-seryl-prolyl-phenylalanyl-nitroargininate (**11**)

1.35 g (1.5 mmole) protected hexapeptide ester [19] was treated with acetic acid—HBr, and the hydrobromide of the hexapeptide ester, coupled with 864 mg benzyloxycarbonyl-arginyl(nitro)-L-pipecolyl-L-pipecolic acid (converted in situ to the azide), and worked up and chromatographed as described for (**9**).

Arginyl-L-pipecolyl-L-pipecolyl-glycyl-phenylalanyl-seryl-propyl-phenylalanyl-arginine triacetate (**12**)

540 mg (**11**) was hydrogenated, chromatographed, and worked up as described for (**2**).

p-Nitrobenzyl benzyloxycarbonyl-arginyl(nitro)-L-pipecolyl-L-pipecolyl-glycyl-phenylalanyl-seryl-L-pipecolyl-phenylalanyl-nitroargininate **13**

1.56 g (1.5 mmole) protected hexapeptide ester [19] was treated with acetic acid — HBr, and the hydrobromide of the hexapeptide ester, coupled with 864 mg benzyloxycarbonyl-arginyl(nitro)-L-pipecolyl-L-pipecolic acid (converted in situ to the azide), and worked up and chromatographed as described for (**9**).

Arginyl-L-pipecolyl-L-pipecolyl-glycyl-phenylalanyl-seryl-L-pipecolyl-phenylalanyl-arginine triacetate **14**

700 mg (**13**) was hydrogenated, chromatographed, and worked up as described for (**6**).

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References

[1] *Schröder, E., K. Lübke:* "The Peptides", Vol. 2. Acad. Press, New York—London, 1966.
[2] *Rudinger, J.:* "Drug Design", Vol. 1. Acad. Press, New York—London, 1971.
[3] *Sheppard, B.* (senior reporter): "Amino-acids, Peptides, and Proteins." A Special Periodical Report. The Chemical Society, London. Vol. 1—9. (1969—1977).
[4] *Merrifield, R. B.:* J. Amer. Chem. Soc. **85**, 2149 (1963).
Merrifield, R. B.: Advances in Enzymology **32**, 221 (1969).
[5] *Katchalski, E., A. Berger, J. Kurtz:* Internat. Symposium on Protein Structure and Crystallography, Madras, 1963.
[6] *Bespalova, Z. D., O. A. Kairov, U. F. Martinov, V. U. Natosky, M. I. Titov, E. I. Sachamtova:* Vest. Leningrad. Univ. Ser. Fiz. Khim. **21**, 157 (1966).
[7] *Chaturvedi, N. C., W. K. Park, R. R. Smeby, F. M. Bumpus:* J. Med. Chem. **13**, 177 (1970).
[8] *Bankowski, K., S. Drabarek:* Acta Polon. Pharm. **33**, 81 (1976).
[9] *Baláspiri, L., Zs. Csapó, M. Fekete, G. Telegdy, K. Kovács:* unpublished results.
[10] *Fairweather, R., J. H. Jones:* J. C. S. Perkin I. **1972**, 2475.
[11] *Vicar, J., M. Budésinsky, K. Blaha:* Coll. Czechosl. Chem. Commun. **38**, 1940 (1973).
Vicar, J., J. Smolikova, K. Blaha: Coll. Czechosl. Chem. Commun. **38**, 1957 (1973).
[12] *Baláspiri, L., A. Török, Zs. Csapó, I. Nógrádi, K. Kovács:* unpublished results.
[13] *Kovács, K., B. Penke, J. Czombos, J. Petres, L. Baláspiri:* Acta Phys. et Chem. Szeged **17**, 61 (1971).
[14] *Baláspiri, L., B. Penke, J. Petres, K. Kovács:* Monatshefte **101**, 1177 (1970).
[15] *Baláspiri, L., Gy. Papp, K. Kovács:* Monatshefte **103**, 581 (1972).
[16] *Nicolaides, E. D., H. A. DeWald, M. K. Craft:* Ann. N. Y. Acad. Sci. **104**, 14 (1963).
[17] *Neubert, K., L. Baláspiri and G. Losse:* Monatshefte **103**, 1575 (1972).
[18] *Baláspiri, L., Gy. Papp, P. Pallai and K. Kovács:* Acta Phys. et Chem. Szeged **20**, 105 (1974).
[19] *Baláspiri, L., B. Penke, Gy. Papp and K. Kovács:* Acta Phys. et Chem. Szeged **20**, 465 (1974).
[20] *Baláspiri, L., J. Lonovics, K. Kovács:* unpublished results.

СИНТЕЗ АНАЛОГОВ БРАДИКИНИНА, СОДЕРЖАЩИХ ПИПЕКОЛИНОВУЮ КИСЛОТУ

Я. Балашпири, Дь. Папп, М. Тот, Ф. Широкман и К. Ковач

Описан синтез, очистка и физические характеристики аналогов брадикинина, содержащих оптически активную — L- и D-пипеколиновую кислоту.